Permethrin-Resistant Human Head Lice, *Pediculus capitis*, and Their Treatment

Kyong Sup Yoon, MS; Jian-Rong Gao, PhD; Si Hyeok Lee, PhD; J. Marshall Clark, PhD; Leon Brown, MS; David Taplin, PhD

**Objective:** To compare the pediculicidal activity of Ovide lotion and its active ingredient, 0.5% malathion, with Nix and its active ingredient, 1% permethrin, in permethrin-resistant head lice.

**Design:** In vitro pediculicidal product and active ingredient comparison. The presence of knockdown resistance–type mutations (T929I and L932F) was validated by DNA sequencing.

**Setting:** University of Massachusetts-Amherst; University of Miami School of Medicine, Miami, Fla; Plantation and Homestead, Fla; and Mathis, Tex.

**Other Participants:** Lice were collected in 3 geographical regions within the United States and in Yamburara, Ecuador, from healthy but infested individuals.

**Intervention:** Within 3 to 6 hours of collection, lice were given a blood meal, exposed to products or active ingredients, and observed at regular intervals.

**Main Outcome Measures:** Percent mortality of lice at regular intervals after exposure to products or active ingredients and presence of T929I and L932F mutations.

**Results:** South Florida lice exhibited a significantly slower mortality response to permethrin compared with susceptible Ecuadorian lice. Ovide and malathion killed permethrin-resistant lice faster than Nix or permethrin. The presence of T929I and L932F in permethrin-resistant south Florida lice was confirmed by DNA sequencing. The population of Texas lice from Mathis was slightly resistant to permethrin and included 13% with resistant genotypes.

**Conclusions:** The presence of the T929I and L932F mutations was confirmed by DNA sequencing in lice collected from children in south Florida that were resistant to the pediculicidal effects of permethrin and the leading permethrin-based head lice product, Nix. Malathion resistance was not observed in this study. The data also show that Ovide killed these same permethrin-resistant head lice approximately 10 times faster than permethrin or Nix.

*Arch Dermatol.* 2003;139:994-1000

---

**PEDICULOSIS,** caused by the human head louse (*HL*) *Pediculus capitis,* is the most prevalent parasitic infestation in children from the United States. Infestations continue to be reported in the United States and in other countries such as those of the United Kingdom (UK), Israel, the Czech Republic, and Argentina despite the many over-the-counter, self-treatment medications available today. In the United States, consumers (nonprescription) treatment products are almost exclusively limited to those that contain the natural botanical active ingredients, the pyrethrins (eg, RID [Pfizer Inc, New York, NY], A-200 shampoo [Hogil Pharmaceutical Corp, Purchase, NY], and Pronto [Del Laboratories, Farmingdale, NY]), or those that contain the synthetic pyrethroid permethrin (eg, Nix [Pfizer/Warner-Lambert, Morris Plains, NJ]), as the active ingredients. These products have been previously reported to be highly effective in the treatment of infestations.

*For editorial comment see page 1061*

It is well established in the published literature that resistance to permethrin exists in certain locations. In the United States, resistance to permethrin has been found in lice from children in Brookline and Cambridge, Mass; Chicopee and Holyoke, Mass; Boise, Idaho; and Plantation, Fla. Although resistance to malathion has been reported in the UK, none has been reported in the United States to date.
Lee et al.\(^1\) reported that US lice were resistant to permethrin and also exhibited knockdown resistance (kdr) in behavioral assays. Knockdown resistance is associated with increased nerve insensitivity and is similar to the kdr noted for DDT, the pyrethrins, and the pyrethroids reported originally in the housefly, Musca domestica.\(^2\) Two point mutations (T929I and L932F) have been found to be associated with permethrin-resistant lice from Florida, Massachusetts, and Bristol, England, but not found in permethrin-susceptible lice from Panama.\(^1\)\(^6\) The T929I mutation functions as a kdr-type mutation in the diamond-back moth, Plutella xylostella.\(^7\) Two point mutations (T929I and L932F) have been found to be associated with permethrin-resistant lice from the UK and substantiate their importance in kdr.\(^2\)\(^1\)

A recently reintroduced prescription treatment (1999), Ovide lotion (Medicis Pharmaceutical Corporation, Phoenix, Ariz), is a pediculicidal product that contains 0.5% pharmaceutical-grade malathion as the active ingredient in a special base. Meinking et al.\(^2\)\(^3\) recently reported that Ovide has superior pediculicidal and ovicidal activity on both susceptible and permethrin-resistant lice compared with the leading permethrin- and lindane-based products. These investigators concluded that Ovide killed permethrin-resistant lice and that these lice were not resistant to malathion.

The purpose of the present study was to confirm and extend the findings of Meinking et al.\(^2\)\(^3\) Standard mortality bioassays determined comparative mortality profiles for both the Nix and Ovide products and their active ingredients. In addition, DNA sequencing determined whether the mutations associated with permethrin resistance were present and what influence they had on mortality.

**Methods**

**Human Head Louse Populations**

The Ecuador test population (EC-HL) was obtained from over 50 children in Yamburara, Ecuador (obtained by D.T.). They had never been exposed to pesticides, including permethrin and malathion, and were considered pediculicide susceptible. A mixed population of all stages and eggs were overnight exposed to the University of Massachusetts-Amherst and placed into a temporary colony fed on humans.\(^1\)\(^6\) The south Florida population (SF-HL) was collected from over 30 infested children in Plantation and Homestead\(^2\) and are permethrin resistant.\(^1\)\(^6\) The Texas population (TX-HL) was obtained from a single heavily infested child (42 lice were obtained) from Mathis, Tex (obtained by L.B.). All collections and administration of informed consent forms were carried out using protocols previously approved by the institutional review board of the University of Massachusetts-Amherst.

**In Vitro Mortality Bioassays**

After collections, the EC-HL were transported to the Pesticide Toxicology Laboratory, Department of Entomology, University of Massachusetts-Amherst; the FL-HL to the Field Epidemiology Survey Team Laboratory; and the TX-HL to a temporary field laboratory in Mathis. All lice were given a blood meal prior to initiation of mortality bioassays by feeding on the investigator's hand.\(^1\)\(^6\) Filter paper and contact bioassays were used to determine lethality of 1% permethrin, 0.5% malathion, Nix (ingredients: 1% permethrin, balsam Canada, cetyl alcohol, citric acid, FD&C yellow #6 fragrance, hydrolyzed animal protein, hydroxyethylcellulose, polyoxyethylene 10 cetyl ether, propylene glycol, stearylalcohol chloride, water, isopropyl alcohol, methylparaben, propylparaben) and Ovide shampoo (ingredients: 0.5% malathion, terpineol, dipentene and pine needle oil in 78% isopropyl alcohol). Filter paper disks (Whatman No. 1) were dipped for 10 seconds into 1% (vol/vol [1 part 10% permethrin in acetone to 9 parts acetone]) permethrin in acetone, 0.5% (vol/vol) malathion in acetone, or Ovide and were subsequently air dried in a dark fume hood for 4 hours. Disks were likewise dipped into Nix for 1 minute and dried in a dark fume hood for 24 hours owing to the slow drying characteristics of this product. Disks were also dipped into neat acetone, dried, and used as nontreatment controls.

The bioassay procedure was conducted according to Lee et al.\(^1\) except that mixed developmental stages (first, second, and third instars and adults) were examined. Log time vs logit mortality regressions were performed (POLO PC; LeOra Software, Berkeley, Calif, 1987) to determine lethal time 50% (LT\(_{50}\)) values. To determine a susceptible or resistant phenotype following 1% permethrin exposure, the lethal time 95% (LT\(_{95}\)) value of the insecticide-susceptible EC-HL was used. Survival beyond the calculated 7.6 hour value of this susceptible strain was used to assess permethrin resistance on a phenotypic basis. The use of timed bioassays at a single dose rather than multiple doses at a single time point is becoming a standard procedure for the assessment of resistance.\(^1\)\(^6\) We have chosen the same concentration that is used in the commercial products for both permethrin and malathion because this is how lice are actually exposed to the active ingredient (at a single concentration over a set time interval). The speed in which permethrin and malathion kills susceptible lice vs the resistant lice is therefore germane in the determination of resistance. Comparisons of the mortality responses due to different test materials were made using the maximum log-likelihood ratio test, which tests the hypothesis of equality of slopes and intercepts of the logit regressions (P = .05 [POLO PC]).

**Determination of the T929I and L932F Mutations**

To determine whether a correlation exists between increased survivorship (due to resistance) and an increasing frequency of kdr-type mutations (T929I and L932F) for permethrin resistance,\(^1\) genomic DNA was extracted from individual SF-HL lice used in the mortality bioassays described previously, which were preserved in 95% ethanol. Extractions were performed using DNeasyol (Molecular Research Center, Cincinnati, Ohio) with a slight modification (addition of 1% polyacryl carrier) to handle small samples according to the manufacturer’s instruction. Genomic DNA was also obtained from a mixed population of permethrin-susceptible and -resistant lice from Mathis (TX-HL) and used as internal quality controls to assure that the DNA amplification and sequencing reactions were successful and capable of identifying lice with and without mutations. Two rounds of polymerase chain reaction (PCR) were performed with nested primers (BL5/Gn/3SP1 for first PCR and 3SP1L/3SP2N for the second PCR; Table 1) to amplify a DNA fragment (approximately 548 to 561 base pairs) containing the S4 to S6 regions of domain II of para-orthologous, voltage-sensitive sodium channel \(\alpha\)-subunit gene of head lice. The presence or absence of kdr-type mutations (T929I or L932F) was determined by DNA sequencing (ABI 377XL; Applied Biosystems, Foster City, Calif) at the Automated DNA Sequencing Facility, University of Massachusetts-Amherst. Computer software, Gene Runner, Version 3.00 (Hastings Software, Bethesda, Md, 1994) was used to analyze and manage sequencing data.
RESULTS

IN VITRO MORTALITY BIOASSAYS

Treatment of the EC-HL with either 0.5% malathion or 1% permethrin resulted in significantly different and substantially reduced survival times compared with the acetone-treated EC-HL as judged by the maximum log-likelihood ratio test ($\chi^2 = 174.8 \ [P < .001]$ and $\chi^2 = 312.9 \ [P < .001]$, respectively) (Figure 1A). Malathion reduced the LT$_{50}$ value 9.0-fold and permethrin reduced the LT$_{50}$ value 5.2-fold compared with the acetone control (Table 2).

Treatment of the SF-HL with 0.5% malathion, likewise, resulted in significantly different and substantially reduced survival times compared with the acetone-treated SF-HL (Table 2). Although treatment of the SF-HL with 1% permethrin also resulted in a significantly different response ($\chi^2 = 159.0 \ [P < .001]$) (Figure 1B), the LT$_{50}$ value was not substantially reduced compared with the acetone treated SF-HL (1.3-fold, Table 2).

The SF-HL (Figure 1B) exhibited significantly slower mortality response to 1% permethrin compared with the EC-HL (Figure 1A) ($\chi^2 = 218.5 \ [P < .001]$). The mortality resistance ratio (RR) based on the LT$_{50}$ values of the SF-HL vs the EC-HL was 3.1 (Table 2) and confirmed that the SF-HL was resistant to permethrin.

The mortality response of the SF-HL (Figure 1B) to 0.5% malathion, however, was not statistically different from the EC-HL (Figure 1A) ($\chi^2 = 2.7 \ [P = .26]$). Although the LT$_{50}$ value of the SF-HL was slightly longer than for the EC-HL, the magnitude of difference was small (RR=1.2) and their confidence limits were overlapped (Table 2). These results confirm that the SF-HL and

---

**Table 1. PCR Primers for the Amplification of a Genomic DNA Fragment of the Para-Orthologous Sodium Channel α-Subunit Gene From the Human Head Louse**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL5'Gn</td>
<td>5'GAGCTCTCAATTGGCCAAATCGTG-3'</td>
</tr>
<tr>
<td>3SP1</td>
<td>5'CTGGTCAGGCGG3TGGGAGCAGA-3'</td>
</tr>
<tr>
<td>5SP1L</td>
<td>5'CCACGTTAAATTTATTAATTTCAA-3'</td>
</tr>
<tr>
<td>3SP5N</td>
<td>5'GATAAACATAGGAGACCGAATT-3'</td>
</tr>
</tbody>
</table>

Abbreviation: PCR, polymerase chain reaction.

**Table 2. Lethal Time Values and Resistance Ratios From Human Head Louse Populations Treated With Various Pediculicidal Compounds and Products**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>EC-HL LT$_{50}$ (95% CI), min</th>
<th>Louse Sample No.</th>
<th>SF-HL LT$_{50}$ (95% CI), min</th>
<th>Louse Sample No.</th>
<th>TX-HL LT$_{50}$ (95% CI), min</th>
<th>Louse Sample No.</th>
<th>RR*</th>
<th>RR†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Permethrin</td>
<td>187 (169-204)</td>
<td>45</td>
<td>577 (538-616)</td>
<td>30</td>
<td>268 (178-339)</td>
<td>33</td>
<td>3.1</td>
<td>1.4</td>
</tr>
<tr>
<td>0.5% Malathion</td>
<td>107 (83-132)</td>
<td>16</td>
<td>129 (79-197)</td>
<td>30</td>
<td>NA</td>
<td>NA</td>
<td>1.2</td>
<td>NA</td>
</tr>
<tr>
<td>Nix (Pfizer/Warner-Lambert, Morris Plains, NJ)</td>
<td>NA</td>
<td>NA</td>
<td>551 (516-587)</td>
<td>22</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ovide (Medicis Pharmaceutical Corporation, Phoenix, Ariz)</td>
<td>NA</td>
<td>NA</td>
<td>57 (35-79)</td>
<td>30</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ovide vehicle</td>
<td>NA</td>
<td>NA</td>
<td>38 (30-46)</td>
<td>15</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Control</td>
<td>965 (934-993)</td>
<td>30</td>
<td>748 (702-790)</td>
<td>30</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; EC-HL, Ecuador test population; LT$_{50}$, lethal time 50%; NA, not applicable; RR, mortality resistance ratio; SF-HL, south Florida test population; TX-HL, Texas test population.

*RR = LT$_{50}$ (SF-HL)/LT$_{50}$ (EC-HL).
†RR = LT$_{50}$ (TX-HL)/LT$_{50}$ (EC-HL).
‡Significantly different from EC-HL (maximum log-likelihood test, P<.05).
§Significantly different from 1% permethrin treatment in SF-HL (maximum log-likelihood test, P=.05).
||Significantly not different from 1% permethrin treatment in SF-HL (maximum log-likelihood test, P>.05).
EC-HL share a similar level of susceptibility to malathion and that the SF-HL lice were not resistant to this pediculicide.

Comparatively, the mortality responses of the SF-HL to 0.5% malathion (Figure 1B) and Ovide (Figure 2) were significantly faster than when treated with 1% permethrin (Figure 1B) ($\chi^2 = 141.9$ [P < .001] and $\chi^2 = 173.0$ [P < .001], respectively) or with Nix (Figure 2) ($\chi^2 = 105.6$ [P < .001] and $\chi^2 = 140.6$ [P < .001], respectively). These findings indicate both 0.5% malathion and Ovide are more efficient in killing permethrin-resistant lice in the SF-HL than 1% permethrin or Nix. The data also show that Ovide kills permethrin-resistant lice from the SF-HL approximately 10 times faster than 1% permethrin and Nix (Table 2).

The mortality response of the SF-HL to Nix (Figure 2) is statistically the same as 1% permethrin (Figure 1B) ($\chi^2 = 1.8$ [P < .4]). Similarly, the magnitude of the difference using the LT50 values was small (LT50 of 1% permethrin-treated SF-HL/LT50 of Nix-treated SF-HL = 1.0) and their confidence limits were overlapped (Table 2).

In the SF-HL, Ovide (Figure 2) exhibited a somewhat faster mortality response (2.3-fold, Table 2) compared with 0.5% malathion (Figure 1B) ($\chi^2 = 61.1$ [P < .001]). Additionally, Ovide vehicle (Ovide without malathion) also elicits lethality. These results suggest that other ingredients (eg, isopropanol and terpenes) in Ovide may have pediculicidal activity. These differences may or may not be clinically meaningful but deserve further investigation.

**LINKAGE OF PERMETHRIN-RESISTANT PHENOTYPES AND KDR-TYPE MUTATIONS**

Two populations were used to establish a linkage between increasing survivorship to 1% permethrin and increasing frequency of the 2 kdr-type mutations. The TX-HL was one of the most permethrin-susceptible US populations assayed to date as judged by its LT50 value compared with that of the SF-HL (2.2-fold more susceptible, Table 2) and by its calculated RR vs the EC-HL (RR = 1.4; Table 2). Additionally, the overlap of its regression line with that of the permethrin-resistant SF-HL/LT50 of Nix-treated SF-HL/LT50 of Nix (Figure 3) is statistically the same as 1% permethrin (Figure 2) ($\chi^2 = 61.1$ [P < .001]). These findings indicate that both 0.5% malathion and Ovide are more efficient in killing permethrin-resistant lice in the SF-HL than 1% permethrin or Nix. The data also show that Ovide kills permethrin-resistant lice from the SF-HL approximately 10 times faster than 1% permethrin and Nix (Table 2).

The mortality response of the SF-HL to Nix (Figure 2) is statistically the same as 1% permethrin (Figure 1B) ($\chi^2 = 141.9$ [P < .001] and $\chi^2 = 173.0$ [P < .001], respectively) or with Nix (Figure 2) ($\chi^2 = 105.6$ [P < .001] and $\chi^2 = 140.6$ [P < .001], respectively). These findings indicate both 0.5% malathion and Ovide are more efficient in killing permethrin-resistant lice in the SF-HL than 1% permethrin or Nix. The data also show that Ovide kills permethrin-resistant lice from the SF-HL approximately 10 times faster than 1% permethrin and Nix (Table 2).

In the SF-HL, Ovide (Figure 2) exhibited a somewhat faster mortality response (2.3-fold, Table 2) compared with 0.5% malathion (Figure 1B) ($\chi^2 = 61.1$ [P < .001]). Additionally, Ovide vehicle (Ovide without malathion) also elicits lethality. These results suggest that other ingredients (eg, isopropanol and terpenes) in Ovide may have pediculicidal activity. These differences may or may not be clinically meaningful but deserve further investigation.

The mortality response of the SF-HL to Nix (Figure 2) is statistically the same as 1% permethrin (Figure 1B) ($\chi^2 = 141.9$ [P < .001] and $\chi^2 = 173.0$ [P < .001], respectively) or with Nix (Figure 2) ($\chi^2 = 105.6$ [P < .001] and $\chi^2 = 140.6$ [P < .001], respectively). These findings indicate that both 0.5% malathion and Ovide are more efficient in killing permethrin-resistant lice in the SF-HL than 1% permethrin or Nix. The data also show that Ovide kills permethrin-resistant lice from the SF-HL approximately 10 times faster than 1% permethrin and Nix (Table 2).

The mortality response of the SF-HL to Nix (Figure 2) is statistically the same as 1% permethrin (Figure 1B) ($\chi^2 = 141.9$ [P < .001] and $\chi^2 = 173.0$ [P < .001], respectively) or with Nix (Figure 2) ($\chi^2 = 105.6$ [P < .001] and $\chi^2 = 140.6$ [P < .001], respectively). These findings indicate that both 0.5% malathion and Ovide are more efficient in killing permethrin-resistant lice in the SF-HL than 1% permethrin or Nix. The data also show that Ovide kills permethrin-resistant lice from the SF-HL approximately 10 times faster than 1% permethrin and Nix (Table 2).

In the SF-HL, Ovide (Figure 2) exhibited a somewhat faster mortality response (2.3-fold, Table 2) compared with 0.5% malathion (Figure 1B) ($\chi^2 = 61.1$ [P < .001]). Additionally, Ovide vehicle (Ovide without malathion) also elicits lethality. These results suggest that other ingredients (eg, isopropanol and terpenes) in Ovide may have pediculicidal activity. These differences may or may not be clinically meaningful but deserve further investigation.

In the SF-HL, Ovide (Figure 2) exhibited a somewhat faster mortality response (2.3-fold, Table 2) compared with 0.5% malathion (Figure 1B) ($\chi^2 = 61.1$ [P < .001]). Additionally, Ovide vehicle (Ovide without malathion) also elicits lethality. These results suggest that other ingredients (eg, isopropanol and terpenes) in Ovide may have pediculicidal activity. These differences may or may not be clinically meaningful but deserve further investigation.
notypic and genotypic determinations. These results indicate a strong correlation between the presence of the T929I and L932F mutations and survivorship beyond 7.6 hours in the 1%-permethrin bioassay, which is indicative of kdr-type permethrin resistance.

**DETERMINATION OF THE FREQUENCY OF KDR-TYPE MUTATIONS**

Lice were randomly selected from the SF-HL used in the mortality bioassays for Nix (Figure 2), 0.5% malathion

<table>
<thead>
<tr>
<th>Pediculicide Louse Sample (N = 33)</th>
<th>Time Died, h</th>
<th>Phenotype* (S or R)</th>
<th>Genotype† T929I</th>
<th>L932F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Permethrin Per1</td>
<td>18</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Per2</td>
<td>13</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Per3-4</td>
<td>12</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Per5-6</td>
<td>11</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Per7-10</td>
<td>7</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Per11</td>
<td>8</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Per12-15</td>
<td>9</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Per16-21</td>
<td>10</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Per22-26</td>
<td>11</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Per27</td>
<td>12</td>
<td>R</td>
<td>Unreadable†</td>
<td>Unreadable†</td>
</tr>
<tr>
<td>Per28-29</td>
<td>12</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Per30</td>
<td>13</td>
<td>R</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Nix§ (N = 9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nix1-3</td>
<td>15</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Nix4</td>
<td>12</td>
<td>R</td>
<td>Unreadable</td>
<td>Unreadable</td>
</tr>
<tr>
<td>Nix5</td>
<td>12</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Nix6-8</td>
<td>10</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Nix9</td>
<td>9</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

Abbreviations: H, heterozygote allele; R, resistant allele; S, susceptible allele.

* Determined by lethal time 95% value (7.6 hours) of 1% permethrin bioassay using the Ecuador test population. A louse that died in 7.6 hours or less was susceptible. A louse that died in more than 7.6 hours was resistant.

† Genotype was assessed by actual DNA sequencing with T929 and L932 being susceptible and I929 and F932 being resistant genotypes.

‡ The DNA sequence cannot be determined owing to the high background in chromatogram.

©2003 American Medical Association. All rights reserved.
Another conducted by Pollack et al.15 owing to several dif-

... those observed for both studies by Meinking et al.2,3 and 
kill times observed in the present study are longer than 
though the overall conclusions are the same, the in vitro 

differences, there is a clear need for standardizing the way in which pediculicidal ef-
fectiveness and resistance are evaluated in vitro. It must 
also be noted that the slower kill times for permethrin 
and Nix may not be clinically relevant as long as they also 
kill all the lice, albeit more slowly, in practice. This co-

... that the presence of kdr-type point mutations and permethrin resis-
tance in this subpopulation. 

Of the 9 randomly selected SF-HL lice treated with 
Nix, 100% (9/9) were phenotypically and 100% (8/8) were 
genotypically resistant, with 1 sample (Nix4) that was 
unreadable (Table 4). These results clearly establish the 
presence of kdr-type mutations and permethrin resis-
tance in this subpopulation. 

Of the 5 randomly selected SF-HL lice treated with 
0.5% malathion and the 7 lice treated with Ovide, 100% 
were genotypically resistant to permethrin (5/5 and 7/7, 
respectively) (Table 5). However, all were assessed to be 
phenotypically susceptible to 0.5% malathion and Ovide 
as judged by the LT50 value of 0.5% malathion on the pe-
diculicidal-susceptible EC-HL (5.1 hours, Figure 1A), and 
all died prior to the 7.6 hour value used to assess pheno-
typic resistance to permethrin, indicating that there is 
no cross-resistance to malathion in permethrin-
resistant lice. Although based on limited numbers, these 
results show that, even in the presence of the kdr-type 
mutations and permethrin resistance, both 0.5% mal-
athion and Ovide provided much faster kill times than 1% 
permethrin or Nix.

We continue to confirm the presence of permethrin-
resistant lice on individuals with pediculosis in the United States. Overall, the SF-HL is resistant to permethrin due to 
kdr-type mutations, but 0.5% malathion and Ovide kill these permethrin-resistant head lice in a manner not signif-
icantly different from that elicited by the insecticide-
susceptible EC-HL. We were also able to demonstrate that 
a prescription-only product, Ovide, was able to kill the 
permethrin-resistant lice at a rate that was approxi-
ately 10 times faster than that observed for Nix. 

Our mortality bioassay data are in agreement with 
findings from 3 other independent studies.2,15,16 Al-
though the overall conclusions are the same, the in vitro 
time killed observed in the present study are longer than 
those observed for both studies by Meinking et al.15 and 
another conducted by Pollack et al.15 owing to several 
differences between the in vitro mortality bioassay tech-
niques used. In view of these differences, there is a clear 
need for standardizing the way in which pediculicidal ef-
ficacy and resistance are evaluated in vitro. It must 
also be noted that the slower kill times for permethrin 
and Nix may not be clinically relevant as long as they also 
kill all the lice, albeit more slowly, in practice. This co-

study that assesses the effectiveness of these products and 
the survivability of permethrin-resistant lice. The pres-
ent work provides the means to carry out such a study.

The combination of mortality bioassays and DNA 
sequence analysis confirms the original findings of Lee 
and coworkers16 that the presence of kdr-type point 
mutations T929I and L932F is highly associated with 
permethrin resistance in field-collected head lice. The 
data presented also confirm and extend the recent find-

... R R R 

... R R R

* Determined by lethal time 95% value (5.1 hours) of 0.5% malathion 
bioassay using the Ecuador test population. A louse that died in 5.1 hours or 
less was susceptible. A louse that died in more than 5.1 hours was resistant.
† Genotype was assessed by actual DNA sequencing with T929I and L932F 
being susceptible and I929 and F932 being resistant genotypes.
‡ Medicis Pharmaceutical Corporation, Phoenix, Ariz.

Accepted for publication February 11, 2003.

This study was supported by grant PHS 1 R01 AI45062-
01A1 from the National Institutes of Health National Insti-
tute of Allergy and Infectious Diseases, Bethesda, Md, and 
by a grant-in-aid from Medicis Pharmaceutical Corpora-
tion, Phoenix, Ariz.

We sincerely thank Lice Source Services, Inc, and Field 
Epidemiology Survey Team members for their assistance in 
louse collections and shipments.
Corresponding author and reprints: J. Marshall Clark, PhD, Department of Entomology, University of Massachusetts, Amherst, MA 01003.

REFERENCES